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Filtration and Utilization of Laboratory-Cultured Bacteria by *Dreissena polymorpha*, *Corbicula fluminea*, and *Carunculina texasensis*

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Abstract. *Dreissena polymorpha* consumed about 6 $\times 10^8$ *Escherichia coli* from 20 ml of artificial pondwater (APW) in 30 min under laboratory conditions. The clearance rate per mussel was 143 ± 25 ml g^{-1} dry tissue min^{-1}. The *E. coli* used in these studies ranged from about 1.7 to 2.9 μm in length. ^{35}S-labeled *E. coli* were used to demonstrate that bacteria-derived nutrients were incorporated into mussel tissue. Electrophoretic analysis of mussel and bacterial proteins on 12% polyacrylamide gels allowed the visual determination of incorporation of labeled amino acids into bivalve proteins and demonstrated that intact bacteria were not simply trapped in mussel tissues. The conversion of bacterial-labeled amino acids into mussel protein was about 26%. Similarly, we demonstrated that *D. polymorpha* can use other bacterial species ranging in size from about 1.3 to 4.1 μm, including *Citrobacter freundii*, *Enterobacter aerogenes*, *Serratia marcescens*, *Bacillus megaterium*, and *B. subtilus*. The ability of *D. polymorpha* to take up *E. coli* was compared with that of two other freshwater mussels, *Corbicula fluminea* and *Carunculina texasensis*. On a mussel-dry-weight basis, *D. polymorpha* cleared bacteria 30 to 100 times faster than *Corbicula fluminea* and *Carunculina texasensis*, respectively. The ability to filter *E. coli* appears to be related to the architecture of the cirri on the latero-frontal cells of the gill. Cirri from *Corbicula* and *Dreissena* are similar in size, but *Dreissena* has a larger gill compared to the tissue dry-weight, and has 10^2 times more cirri than found in *Corbicula*. *Carunculina*, the unionid representative, has smaller and fewer cirri, and has relatively limited ability to capture *E. coli*.

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Abbreviations: APW—artificial pondwater; TCA—trichloroacetic acid; PAGE—polyacrylamide gel electrophoresis; SDS—sodium dodecyl sulfate; SEM—scanning electron microscopy

Introduction

Dreissena polymorpha (Pallas) has successfully colonized much of the Great Lakes region of North America. It is currently achieving the same success in the Ohio, Tennessee, and Mississippi river drainages (unpubl. obs.). This organism can filter large amounts of water in a relatively short period, eliminating or greatly reducing the abundance of zooplankton and phytoplankton (Stanczykowska *et al.*, 1976; MacIsaac *et al.*, 1992; Leach, 1993; Bunt *et al.*, 1993). Previous reports indicate that *D. polymorpha* selects food particles in the size range 15–40 μm (Ten Winkel and Davids, 1982), and filters particles >2 μm with almost 100% relative efficiency (Jørgensen *et al.*, 1984). Capture of particles ranging in size from 0.7 μm (Sprung and Rose, 1988) to 750 μm (Ten Winkel and Davids, 1982) has been reported.

Initial capture of particles occurs on the gills of eulamellibranch bivalves and is mediated by the ciliary mechanical systems associated with the gill filaments. While all eulamellibranch gills are organized into filaments, the structure and organization of specialized ciliary appendages associated with the filaments vary from species to species (Atkins, 1938; Morton, 1983). All have lateral ciliated cells that are generally believed to be responsible for moving water through the gill. The latero-frontal cells are positioned between the frontal surface of the filament and the lateral ciliated cells. The ciliary appendages on these cells range from simple cilia in some species, to longer, stiffer cilia in other species, to fused plates of cilia in still others. In bivalves with cirri, the number of cilia per plate varies with species: 11–12 fused cilia in *Carunculina tex-*

asensis, 22–26 in *Mytilus edulis*, and 38–42 fused cilia in *D. polymorpha* (Atkins, 1938; Moore, 1971; Owen, 1974). These structures, together with even more distally located abfrontal and frontal cilia, act in concert to capture and move particles. The ciliated structures, the water currents they produce, and the mucus produced by the gills and palps move food particles toward the mouth (Beninger *et al.*, 1992; 1993; Ward *et al.*, 1993). On the basis of structure alone, some investigators have indicated that cirri or latero-frontal cilia act as a mechanical filtering device that can explain particle trapping in various bivalve species (Owen, 1974; Owen and McCrae, 1976; Silvester and Sleigh, 1984). However, the actual mechanism of particle capture remains a topic of disagreement. Jorgensen (1976, 1982, 1989) has argued that such descriptions fail to account for fluid movements and the complex currents associated with the gill. Recent endoscopic studies directly demonstrated that mucus is involved in transporting particles after their capture by the gill (Beninger *et al.*, 1992; Ward *et al.*, 1993).

In this study, we describe controlled laboratory experiments aimed at assessing how well *D. polymorpha* filters bacteria, and we compare the results to those for other freshwater mussels. The experiments tested the ability of *D. polymorpha*, *Carunculina texasensis* (a unionid representative), and *Corbicula fluminea* to utilize laboratory-cultured *Escherichia coli* as a sole nutrient source. Under laboratory conditions *D. polymorpha* showed rapid filtration and incorporation of ^{35}S-labeled *E. coli*. The clearance of bacteria from artificial pondwater (APW) was faster in *Dreissena* than in either *Corbicula fluminea* or *Carunculina texasensis*. These differences in filtration in laboratory studies suggest that the ability to use natural-sized bacteria in the environment may differ substantially among these freshwater species.

Materials and Methods

Animals

Dreissena polymorpha [range 17–25 mm length; 1.225 ± 0.027 g total live weight (mean ± SE); 0.016 ± 0.000 g dry tissue; n = 160]; was collected from the Mississippi River from screens at the Dow Chemical Plant in Plaquemine, Louisiana. The unionid *Carunculina texasensis* (23–26 mm length; 2.453 ± 0.073 g total live weight; 0.090 ± 0.006 g dry tissue; n = 10) was collected, under permit, from a pond in Baton Rouge, Louisiana; and *Corbicula fluminea* (23–25 mm length; 7.871 ± 0.233 g total live weight; 0.368 ± 0.012 g dry tissue; n = 36) was collected from the Tangipahoa River in southern Mississippi. All species were kept under laboratory conditions in aerated artificial pondwater (APW; 0.5 NaCl, 0.4 $CaCl_2$, 0.2 $NaHCO_3$, 0.05 KCl in mM) with *Dreissena* in APW containing 0.2 mM Mg_2SO_4 (Dietz *et al.*, 1994). Animals were maintained unfed in the laboratory for five days before use.

Labeling of bacteria

Escherichia coli JM83 (Messing, 1979) was used for most of the experiments. For ^{35}S-labeling, *E. coli* were grown in a chemically defined medium containing 5 g glucose, 810 mg NH_4Cl, and 82 mg $MgCl_2 \cdot 7H_2O$ per liter of 0.05 M potassium phosphate buffer, pH 7.2. Added to this was 5 ml of a trace salts solution containing $CaCl_2 \cdot 2H_2O$ (2 g), $MnSO_4 \cdot H_2O$ (1 g), and $FeSO_4 \cdot 7H_2O$ (0.5 g) dissolved in one liter of 0.1 M HCl. For growth of *E. coli* JM83, it was necessary to add 20 μg/ml L-proline and 5 μg/ml thiamine, final concentrations, to the medium. The carbon source, trace salts solution, L-proline, and thiamine were sterilized separately from the rest of the medium. To label the bacteria, *E. coli* were grown at 37°C with shaking aeration for at least four generations in the above medium containing 5 μCi/ml carrier-free $Na_2{}^{35}SO_4$ (Dupont NEN). The final cell density of cultures was approximately $1.3–2 \times 10^9$ bacteria/ml. Labeled bacteria were collected by centrifugation, washed once in the growth medium without carbon source, and stored in APW at a concentration of 3×10^9 bacteria/ml. The cells were stored on ice in pondwater until use. Following this initial transfer, the bacteria did not experience any additional osmotic shock and survived for weeks. *E. coli* grown in this medium were 2.3 ± 0.6 μm long and 0.9 ± 0.1 μm wide (n = 50) and did not clump.

Incorporation of ^{35}S during growth of *E. coli* was measured with a liquid scintillation counter. The bacteria were precipitated in 10% trichloroacetic acid (TCA) and collected on a glass fiber filter. Greater than 70% incorporation of the label was routine.

To determine if filtration of *E. coli* by *D. polymorpha* was novel for freshwater bivalves or whether other bacterial species would be similarly filtered, several other bacteria differing in size (1.3–4.1 μm in length) and shape were tested. A similar ^{35}S-labeling protocol was used to label *Citrobacter freundii*, *Enterobacter aerogenes*, *Serratia marcescens*, *Bacillus subtilus*, and *B. megaterium*. The concentration of bacteria in all suspensions was determined by direct microscopic count using a hemocytometer. Cell dimensions for labeled bacteria were measured from photomicrographs.

Escherichia coli *feeding experiments*

All feeding experiments were carried out in individual containers (test tubes) aerated for the duration of the experiment. Individuals of similar size were selected by weighing, then placed in separate test tubes containing 20 ml of APW. The experiment was started by the addition of bacteria as soon as the bivalves began siphoning.

Siphoning typically began within 10 min of placing animals in the test tube containing pondwater.

Each test tube had 3×10^7 bacteria/ml representing about 1.7×10^5 dpm ^{35}S/ml (200 μl stock bacterial cell suspension). Each day the *E. coli* stock solution (in APW and held on ice) was centrifuged and resuspended in APW. The discarded supernatant was assayed for ^{35}S. Using this assay procedure, we found that *E. coli* did not deteriorate, and that all ^{35}S (>99%) added to an experimental tube was associated with intact bacteria in the APW and not with breakdown products in the supernatant. Control tubes without bivalves received labeled bacteria, were aerated for 20 min, and analyzed. The bath solutions from these tubes were passed through a Millipore filter (0.22 μm) to trap the bacteria. Virtually all of the label in each tube (99.5%) was on the filter, while the supernatant contained 1319 ± 85 dpm/ml (n = 15). Thus, <0.5% of the radioactivity was in the non-particulate material of the assay medium (similar results were obtained by centrifuging the assay medium; less than 1% of the radioactivity remained in the supernatant).

The bivalve filtration studies were initiated by collecting a sample (t_0) of the bath exactly 45 s after inoculation of the pondwater with bacteria. This time interval was required for mixing and was previously determined both visually (methylene blue) and by tracking the distribution of labeled bacteria in test tubes without an animal present. Individual test tubes were usually sampled initially and at the end to avoid disturbing the animals, with final samples collected after 5 to 90 min, depending on the bivalve species. The final sample was taken by mixing the tube and taking a 100-μl sample. For all samples, ^{35}S radioactivity was determined with a liquid scintillation counter (Wiegman *et al.*, 1975). Additional controls for these experiments consisted of dried shells or rinsed, formalin-fixed whole animals placed in individual containers and handled as described above. In none of the controls was radioactivity significantly reduced in the bath. At the conclusion of the experiments, animals were removed from their shells and dried overnight to constant weight at 90°C. Radioactive ^{35}S adsorbed to control (fixed) animal tissue or shell was less than 100 dpm. Incorporation of label was not detectable in the control tissue analyses (see below).

At the end of some experiments, each animal was removed from the tube and rinsed 2–3 times in >500 ml pondwater containing no label, then placed in a separate beaker containing at least 150 ml of APW. The label was allowed to be incorporated into the animal for 48 h, with two additional changes (rinsed 3 × 500 ml) of APW each day. Usually several hundred dpm/ml were released into the APW by an animal during a 12-h period. After 48-h, each animal was removed from the bath and rinsed several times in APW and a blood sample taken by pericardial puncture (Fyhn and Costlow, 1975). Blood sampling location was previously determined by dissection of *D. polymorpha* to identify appropriate landmarks. A syringe needle (15.9 mm, 26 ga) was inserted between the valves between the inhalant and exhalant siphons and into the pericardial cavity located in the vicinity of the posterior margin of the hinge (Dietz *et al.*, 1994). To collect >100 μl of blood, equal to 10–20% of the animal's wet weight, the syringe needle had to be rotated to prevent tissue from occluding the needle orifice. The osmolality of the APW was 4 mOsm and the water contained about 300 dpm/ml radioactivity at the time of collection of blood from the bivalves. Measured osmolarity of the blood ranged between 40 and 42 mOsm, within the normal range previously reported (Dietz *et al.*, 1994), and radioactivity in the blood was 1 to 4 orders of magnitude higher than that found in APW. These data suggest little if any contamination of blood samples by mantle cavity fluid.

Animals were dissected free of the shell and the tissue was dried overnight at 90°C and weighed. The tissue was homogenized in 3% TCA, and the TCA-precipitable pellet was washed twice in TCA and re-pelleted. The final supernatant was discarded and the pellet dissolved in 1 *M* NaOH. A sample of digested tissue was assayed for radioactivity and an aliquot analyzed for protein content using a BioRad protein determination procedure. This allowed us to determine the amount of ^{35}S incorporated following the feeding experiments. The 48-h rinse in large volumes of APW was used to allow passage of any bacteria that might have remained in the gut, and also allowed time for incorporation of radioactive label into clam proteins.

Variations in the basic experimental protocol included experiments designed to compare clearance rates in different volumes of bath (with constant bacterial concentration) and at different bacterial concentrations (while keeping bath volume constant). Volumes tested were 20, 60, and 120 ml. Even with the small 20-ml bath volume, the reduction of bacterial radiolabel followed first-order exponential kinetics. The use of a small volume reduced the amount of radiolabeled waste fluid produced. In addition, we maintained the bath at a constant 20 ml while increasing the number of bacteria present. The latter experiments were designed to assess maximal bacterial clearance under laboratory conditions.

Finally, in an attempt to assess whether repeated exposure to bacteria led to satiation, we exposed some animals to 6×10^8 *E. coli* in 20 ml of bath, assayed bacterial disappearance from the medium over 30 min, waited 30 min and added another 6×10^8 cells; this process was repeated until we determined that the animals were no longer removing bacteria from the bath at the initial rate.

Electrophoretic analysis of ^{35}S-labeled proteins

To demonstrate the assimilation of bacterial sources of sulfur-containing amino acids by *D. polymorpha*, a few animals were dissected directly into liquid nitrogen after 48-h pulse labeling. Each animal, as well as a separate sample of the ^{35}S-labeled *E. coli* (10^9 cells), was placed in 400 μl of ice-cold buffer (10 m*M* Tris-HCl, 1 m*M* EDTA, 10 m*M* NaCl, pH 7.8) with protease inhibitor, 0.7 mg/ml phenylmethylsufonyl fluoride. The samples were partially homogenized by sonic disruption using the microtip probe of an ultrasonic processor (Model W220, Heat Systems-Ultrasonic, Inc.). Samples received four 10-s bursts at a power setting of 2 and were cooled on ice for 3 min between treatments. Following sonication, the samples were centrifuged at 16,000 × *g* for 3 min, and the supernatant fluid was collected on ice. For each cell-free extract, the TCA precipitable radioactivity was determined, and the protein content was assayed. Proteins in the cell-free extracts were separated by electrophoresis on a 12% polyacrylamide gel (PAGE) according to the method of Laemmli (1970). *D. polymorpha* samples contained approximately 100,000 dpm in 60 μg of protein. Following electrophoresis, samples were visualized by staining with Coomassie brilliant blue and by autoradiography with Kodak X-OMAT AR film. Molecular weight standards (Broad range SDS-PAGE Standards, BioRad Laboratories) stained with Coomassie brilliant blue were used to define the banding patterns of the samples.

Cirral structure

Differences in cirral structure and distribution were examined and described using scanning electron microscopy (SEM). Tissue fixation followed modification of previously described procedures (Richard *et al.*, 1991). Animals were fixed for 1 h in 2% glutaraldehyde in phosphate buffer (35-60 mOsm as appropriate to match blood osmolality of the bivalve species). Gills were excised and exposed to glutaraldehyde for an additional hour, rinsed in buffer, and postfixed in 1% osmium tetroxide. Gills were dehydrated in an ethanol series, critical-point dried, mounted on aluminum stubs using carbon tape, and sputter-coated with gold/palladium (20 nm). Gills were examined using a Cambridge 200 scanning electron microscope. For calculations of cirral size and gill surface areas it was necessary to measure the shrinkage associated with tissue processing for SEM examination. For these purposes, several gills from each species were carefully excised and photographed using a dissecting microscope. The tissue was fixed and critical-point dried as described above, then re-photographed to allow determination of tissue shrinkage under our tissue preparation procedures.

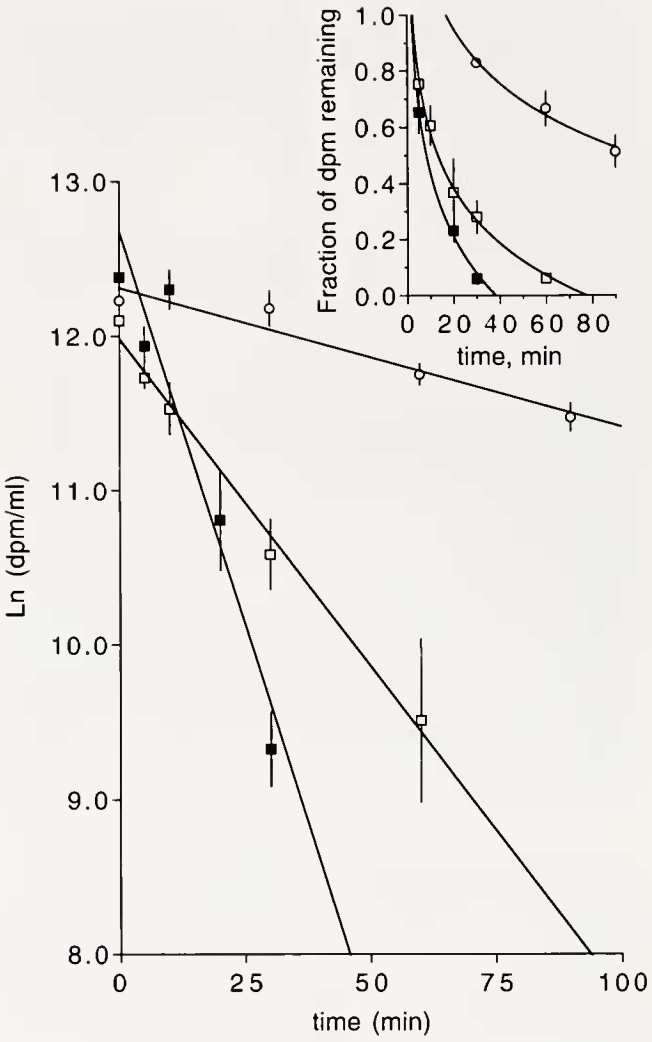

Figure 1. Inset is the time-dependent removal of *Escherichia coli* from pondwater by *Dreissena polymorpha* (filled squares), *Corbicula fluminea* (open squares), or *Carunculina texasensis* (open circles). The vertical lines represent ±1 standard error. The slope of the line of the time-dependent natural logarithmic transformation of radioactive bacteria concentration in the APW is the rate constant (min^{-1}). Each mussel was placed in 20 ml of pondwater containing 6×10^8 bacteria labeled with ^{35}S. Each point represents the mean ± standard error for at least 10 separate animals. The $t_{1/2}$ was 7.1, 16.5, and 77.0 min for *D. polymorpha*, *Corbicula fluminea*, and *Carunculina texasensis*, respectively.

Calculations and statistical analysis

Disappearance of radioactive label from the bath was exponential and followed first-order rate kinetics (rate constant = $\ln(D_0/D_t) \cdot t^{-1}$, D was the dpm/ml at times 0 and t). The half time was calculated from the expression $t_{1/2} = \ln(2)/(\text{rate constant})$. Bacterial clearance rate (C, ml min^{-1}) for each mussel was calculated using the equation $C = V/t \cdot \ln(D_0/D_t)$, where V is volume in ml, D_0 and D_t are bacteria radiolabel concentrations defined above (Riisgård, 1988). Weight-specific clearance (dry soft-

Table I

Rate of ^{35}S-labeled E. coli *uptake by three species of freshwater bivalves*

Bivalve species	*n*	Dry tissue, mg	Rate constant, min^{-1}	Clearance, ml g^{-1} dry tissue min^{-1}
Dreissena polymorpha	20	16 ± 1^c	-0.098 ± 0.011^a	143.1 ± 24.6^a
Corbicula fluminea	32	368 ± 12^a	-0.043 ± 0.002^b	4.4 ± 0.6^b
*Carunculina texasensis**	10	90 ± 6^b	-0.009 ± 0.002^c	1.3 ± 0.2^c

Data expressed as mean ± standard error. Values within a column having different letters were significantly different ($P < 0.01$) using an unpaired Student's *t*-test.

* The same animals were sampled repeatedly at 30-min intervals for 90 min.

tissue mass) data were expressed as ml g^{-1} dry tissue min^{-1}. In some cases we determined the rate constants and calculated clearance from a linear regression of ln(dpm/ml) as a function of time to compare rate constants or to calculate clearance between species or experimental treatments. Differences between species or treatments were determined by ANOVA and were considered significant if $P < 0.05$. Fisher's protected least significant difference (PLSD) was used to determine differences between average values within a group having a significant ANOVA. Differences between regression slopes were determined using Student's *t*-test.

Results

Filtration of E. coli *by freshwater bivalves*

Freshwater bivalves removed *E. coli* from pondwater with first-order exponential kinetics (Fig. 1). The size of the cultured *E. coli* was 2.3 ± 0.6 μm long and 0.9 ± 0.1 μm wide. The bacterial concentration used in these experiments was approximately 3×10^7 per ml in 20 ml APW, and 10–36 animals of each species were used to obtain the average values. The slope of the lines represents the rate constants for removal of bacteria by the different bivalve species (Table I). The rate constants differed statistically ($P < 0.01$) for each species, and *D. polymorpha* had the highest rate and the shortest $t_{1/2}$ (Fig. 1). On a dry weight basis, clearance rates also were highest for *D. polymorpha* and lowest for *Carunculina texasensis*. The rate of clearance exhibited by *D. polymorpha* was 30–100 times greater than that of the other two species.

Incorporation of radioactive bacterial nutrients in bivalve proteins

Disappearance of radiolabel from the bath indicated that the mussels were capable of removing the particles from the water column, but did not necessarily indicate that the particles and their associated nutrients were assimilated. Mussels hold food particles in their digestive tube and perhaps in the mantle cavity for days without digesting them. In addition, we have observed living algae escaping into the water column from pseudofeces released by unionids two days after feeding (S. J. Nichols, pers. comm.; unpub. obs.). However, ^{35}S label was accumulated into the body fluids of the animals 48 h after the pulse feeding (Table II). Given the amount of radiolabel observed in the blood of the animals, it was unlikely that the blood samples were contaminated by the APW. The APW bath containing each *D. polymorpha* had only 302 ± 35 dpm/ml ($n = 5$) at the end of the 48-h period. Each of these bivalve species had label in the blood, and this fluid is presumably maintained in a sterile condition by the animal. Thus, label was likely to be attributable to dissolved ^{35}S-containing matter (amino acids, polypeptides, etc.). Further, 48 h after *Dreissena* were exposed to bacteria, a few animals were fed *Chlorella*. After algal feeding (30 min), the gut tubule was visibly green, and was dissected from the animal for measurement of radioactivity. Only bacteria in the gut contents would be radiolabeled and detected by scintillation counting, and in no case were counts above background recorded.

To determine whether the radioactivity in the mussels was in the form of bacterial protein or had been converted into mussel protein, we used PAGE analysis to compare mussel tissue proteins with *E. coli* proteins. The presence of *E. coli* proteins in the PAGE gels of mussel tissue would indicate that intact bacteria were associated with the mussel body. Figure 2, an autoradiograph of a corresponding

Table II

^{35}S accumulated in the blood of freshwater mussels 48 h after they were fed ^{35}S-labeled E. coli *(3×10^7 bacteria/ml) for 60 min*

Bivalve species	Blood, dpm/ml
Dreissena polymorpha	835070 ± 224170^a
Corbicula fluminea	145694 ± 51875^b
Carunculina texasensis	1840 ± 387^c

Mean ± standard error, $n = 5$ for each species. The means with different letters are significantly different using the unpaired Student's *t*-test ($P < 0.05$).

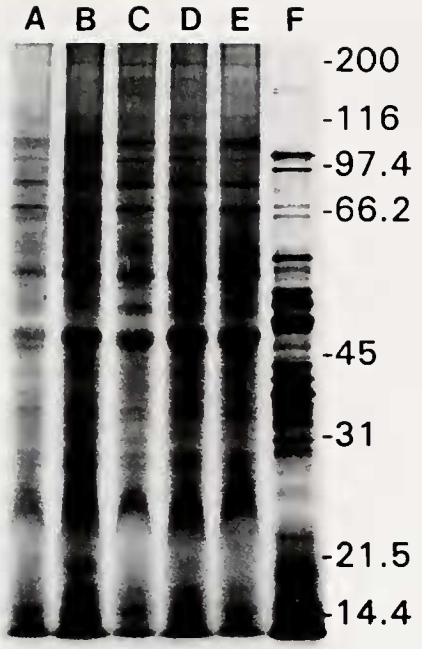

Figure 2. Autoradiograph of a 12% polyacrylamide gel used to electrophoretically separate proteins isolated from the homogenized whole body of *Dreissena polymorpha*. Mussels were allowed 48 h to assimilate ^{35}S-labeled *Escherichia coli* after a 20-min feeding (individual mussels represented in lanes A–E). Lane F represents the solubilized protein fraction of the ^{35}S-labeled *E. coli* used in the feeding experiments. Molecular weight markers were located from the stained gel. Note the similarity between individual *D. polymorpha* (lanes A–E) and the distinctive difference between these lanes and the *E. coli* lane. There was no evidence for the presence of any of the recognized *E. coli* proteins in the *D. polymorpha* lanes (the heavy *E. coli* bands at about 50 kDa and 95–100 kDa were not present in lanes A–E). Conversely, many of the major labeled proteins in the mussel tissue do not appear in the *E. coli* band.

polyacrylamide gel, does not show any overlap between *E. coli* proteins (lane F) and label incorporated into mussel proteins (A–E, each lane representing an individual *D. polymorpha*). The incorporation of ^{35}S into mussel proteins indicated the assimilation of bacterial components; *E. coli* proteins were not present in the bivalve samples.

The lack of bacterial contamination allowed us to determine the incorporation characteristics of nutrients into mussel tissue. Several individuals of each species were allowed to feed on labeled bacteria, removed to pondwater (500 ml, changed twice daily) containing no bacteria or label, and allowed to assimilate label for 48 h following the pulse exposure to radiolabeled bacteria. Whole animals were homogenized, then precipitated in TCA, and the precipitate was dissolved in 1 *M* NaOH. The ^{35}S incorporated into macromolecules/total ^{35}S removed from the bath was 0.29 ± 0.02 (n = 4), 0.23 ± 0.07 (n = 4), and 0.28 ± 0.02 (n = 5) for *D. polymorpha*, *Corbicula fluminea*, and *Carunculina texasensis*, respectively. Thus, regardless of mussel species, about 26% of the label that disappeared from the bath was incorporated into mussel protein 48 h after the pulse feeding experiment. There were no significant differences between animal species in the proportion of ^{35}S assimilated into mussel protein from *E. coli*. Formalin-fixed control tissue had no detectable radioactivity.

Volume of water cleared of bacteria

Increasing the volume of fluid available for clearance by *D. polymorpha* while maintaining a constant concentration of bacteria per milliliter allowed us to determine clearance rates over different volumes, but with differing quantities of total bacteria present in the APW (Fig. 3). The rate constants (slope) for the different volumes were 20 ml, −0.098 ± 0.01 min^{-1}, 60 ml, −0.029 ± 0.001 min^{-1}; 120 ml, −0.009 ± 0.001 min^{-1}. These slopes were significantly different from each other ($P < 0.01$). When expressed as clearance (ml g^{-1} dry tissue min^{-1}), the values were 20 ml, 143 ± 25; 60 ml, 189 ± 26; and 120 ml, 113 ± 11. These weight-specific clearance rates were not significantly different from each other ($P > 0.05$). Because *D. polymorpha* in this data set weighed about 15 ± 1 mg (n = 55), the animals cleared 1.7–2.8 ml animal^{-1} min^{-1} under all experimental conditions, or roughly 2.4–4 liters of water per day for an average animal (about 8.9 l g^{-1} dry tissue day^{-1}).

By varying the concentration of *E. coli* in a constant bath volume and determining the time-dependent (5–60 min) removal of particles from the suspension, we calculated the average clearance rates (Fig. 4). These data

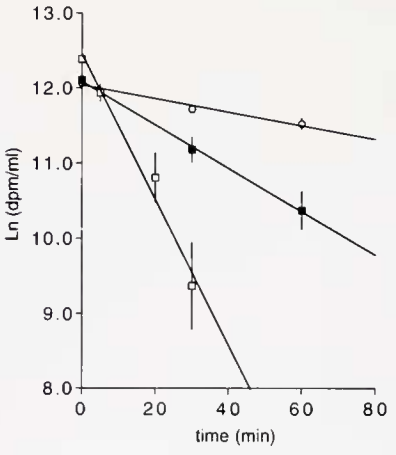

Figure 3. Clearance of ^{35}S-labeled *Escherichia coli* by *Dreissena polymorpha* from different volumes of pondwater containing 3 × 10^7 bacteria/ml. Open squares indicate experiments in 20 ml volume, filled squares in 60 ml, and open circles in 120 ml of pondwater. The vertical line indicates ±1 standard error. The slopes of the lines were significantly different ($P < 0.01$) but when multiplied by the bath volume and normalized to dry tissue weight the clearance values ranged from 113 to 189 ml g^{-1} min^{-1} and were not significantly different ($P > 0.05$).

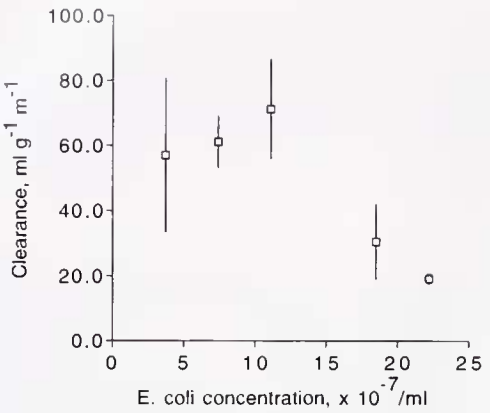

Figure 4. Clearance of *Escherichia coli* from 20 ml of artificial pondwater by *Dreissena polymorpha* as a function of bacterial concentration. Note that bacterial clearance appeared to be approximately constant between 3.7 and 11.1 $\times 10^7$ bacteria/ml but was reduced at higher bacteria concentrations. The vertical lines represent ±1 standard error.

indicate that *D. polymorpha* had a relatively constant rate of bacterial clearance when exposed to bacterial concentrations ranging from 3.7 to 11 $\times 10^7$ bacteria/ml, but at concentrations greater than 18 $\times 10^7$ bacteria/ml the apparent rate of bacterial filtration was significantly reduced ($P < 0.01$).

When bacteria concentration was kept at or below 3 $\times 10^7$ bacteria/ml, no pseudofeces were observed over 60 min. When higher concentrations of bacteria were used, pseudofeces production was visually observed after roughly 60 min of exposure. Thus at the higher bacteria concentrations, pondwater was cleared of radioactivity but some of the label was deposited in pseudofeces rather than accumulated by the animal.

Maximal uptake of bacteria by D. polymorpha

In a few experiments, we followed the disappearance of *E. coli* from the bath under the standard conditions described in Figure 1, but allowed the mussel to remain in the container and added another aliquot of bacteria to the APW after 30 min. The bath was sampled at t_0 and t_{30} to measure isotope uptake. This procedure was repeated until the animal was no longer removing bacteria at the initial rate. Under these conditions five separate feedings were required before the removal of bacteria by the mussel was reduced (data not shown).

Clearance of other bacterial species

To determine whether bacterial species other than *E. coli* could be filtered, specimens of *D. polymorpha* were exposed to a variety of laboratory strains of several bacterial species of different sizes (0.8–1.1 μm width, 1.3–4.1 μm length), following the protocol used to produce Figure 1. Table III indicates the rates of clearance of the bacterial species by *D. polymorpha*, and the dimensions of each species including *E. coli*. In all cases except one, 55–91% of the bacterial cells were removed from the bath in 30 min and the calculated clearance rates were 50–80 ml g^{-1} dry tissue min^{-1}. The exception was *Bacillus megaterium*, which formed large chains of bacteria. Some of these bacterial chains settled to the bottom of our experimental containers and were not available in the APW for the mussel to filter. In this case, the corresponding clearance rate was significantly lower than for the other bacterial species.

Effects of laboratory storage of mussels on bacterial uptake

Separate experiments were performed on animals acclimated to the laboratory for various periods. Clearance rates of *E. coli* by *D. polymorpha* were not significantly different whether the mussels were tested 18 h after they were collected from the Mississippi River or after they had been stored in the laboratory, unfed, for a week: 105 ± 17 (n = 10) versus 108 ± 24 (n = 10) ml g^{-1} dry tissue min^{-1} 18 h after collection and one week later, respectively. Furthermore, these clearance rates were not significantly different from that of *D. polymorpha* maintained in the laboratory, unfed, for several weeks.

Cirral structure

Briefly, the cirrus organelle is composed of two plates of fused cilia and beats from a flexed to an extended position (Moore, 1971; Owen, 1974). In the flexed position the cirri bend up over the frontal surface of the filament. In the extended position, the cirri lie in the interfilament

Table III

Mean cell dimensions ($n > 50$ for each bacterium) for bacteria used in Dreissena polymorpha *studies measuring the clearance from 20 ml APW*

Bacterium species	Cell dimensions (L × W) μm	Dry tissue, mg	Clearance, ml g^{-1} dry tissue min^{-1}
Citrobacter freundii	1.3 ± 0.3 × 0.8 ± 0.1	12 ± 1	64 ± 12 (9)[a]
Enterobacter aerogenes	1.5 ± 0.3 × 0.9 ± 0.1	17 ± 2	50 ± 10 (9)[a]
Serratia marcescens	1.8 ± 0.5 × 0.8 ± 0.1	13 ± 1	52 ± 12 (8)[a]
Escherichia coli	2.3 ± 0.6 × 0.9 ± 0.1	18 ± 4	80 ± 11 (11)[a]
*Bacillus megaterium**	3.6 ± 1.0 × 1.1 ± 0.2	15 ± 1	14 ± 1 (9)[b]
Bacillus subtilus	4.1 ± 0.8 × 0.9 ± 0.2	14 ± 1	59 ± 6 (9)[a]

Mean ± standard error (n). The chains did not remain in suspension during the course of the experiment. Clearance values with different letters are significantly different using Fisher's PLSD test.

* Bacteria in this culture were present in chains with an average length of 14.5 ± 3.7 μm.

space opposing the cirri from the adjacent filament. When extended, each cirrus, along with its adjacent cirri, forms a filtration trap that does not allow movement of particles down into the ostia of the gill. Trapped particles are passed to the frontal cilia when the cirri bend into their flexed position. Differences in the structure of the cirri of *D. polymorpha, Corbicula fluminea,* and *Carunculina texasensis* are shown in Figure 5. The free tips of the cirral cilia form the filtration traps, and the spaces between ciliary tips are in the 0.5-μm range for *Dreissena.* Note the smaller cirral structure of *C. texasensis* compared with the other two species.

Even with an osmotically balanced fixative, gill shrinkage in *Carunculina texasensis* was 16.6% (dorsal-ventral, long axis of the filament) and 38.9% (anterior-posterior); 14.4% and 33.4% in *Corbicula fluminea;* and 23.0% and 25.2% in *D. polymorpha.* In *Carunculina* and *Corbicula* the differential shrinkage between length and width is due to the relatively rigid connective tissue support. *Carunculina* manifests this support as calcified chitinous rods supporting the filaments. *D. polymorpha* shows less support, and shrinkage was more uniform.

Discussion

Dreissena polymorpha was capable of filtering and ingesting large numbers of laboratory-cultured bacteria from the water column. On an individual animal basis with no standardization for size differences among animals, *Corbicula fluminea* cleared *E. coli* at a significantly lower rate ($P < 0.01$) than did *D. polymorpha,* but at a higher rate than did *Carunculina texasensis.* When standardized on the basis of dry tissue weight, the bacterial clearance rate was 30–100 times higher in *D. polymorpha* than in the other two bivalve species. Standardization on the basis of dry tissue weight provides a convenient normalization for clearance study comparisons. However, surface area of the gill is likely to be physiologically important with regard to the actual mechanism of particle capture. Both *Corbicula fluminea* and *Carunculina texasensis* had gill surface areas that were about 1.5 mm^2/mg dry tissue, whereas *D. polymorpha* had about 14 mm^2/mg dry tissue (Table IV). When gill surface area was used as a normalizing factor, it was apparent that the dreissenid species had a greater ability to capture *E. coli* than did the other two species. A regression analysis comparing *E. coli* clearance from the APW with cirri g^{-1} dry tissue among the bivalve species was highly significant ($r = 0.998$; $P < 0.05$), with 100–200 times more cirri in *D. polymorpha* than in the other bivalves.

In addition to gill surface area, another major difference among these three species was the complexity of the laterofrontal cirri associated with the gill filaments. Gill cirri lie between the lateral ciliated cells and the surface of the filaments. The lateral ciliated cells and perhaps the musculature in eulamellibranch gills provide the force for water movement. Although there is considerable debate on the exact mechanism of particle capture (hydrodynamic vs. direct mechanical contact; see Nielsen *et al.,* 1993), the movement of cirri, frontal and abfrontal cilia are apparently coordinated to intercept, capture, and move particles. Recent endoscopic work indicates that ciliary activity, mucus, and water current are important in transporting particles toward the mouth once they are captured (Beninger *et al.,* 1993; Ward *et al.,* 1993). When the cirri are extended, they are directly in the path of water flow between the filaments. The cirri are positioned to trap particles and direct them toward the filament apex and the frontal cilia when the cirri are flexed (unpub. obs.). In the extended position, cirri form an effective filtration "trap" or "net." Unfortunately, the words *trap* and *net* may seem to exclude the possibility of considering water current generation by the cirri as part of the filtration mechanism or "trap." However, if the term is defined to mean "barrier" or "intercepting unit" then the comments here are compatible with previous endoscopic and microscopic observations of particle transport and particle "bouncing" (Jørgensen, 1976; Ward *et al.,* 1993).

The scanning electron micrographs demonstrate clearly that the cirri of *D. polymorpha* and *Corbicula fluminea* are more complex than those of *Carunculina texasensis.* The cirri in the first two species are composed of two ciliary plates containing as many as 42 cilia per plate (unpub. obs.). The cirri of *Carunculina* are less complex, consisting of 11–13 cirri per plate, and not as long or as rigid as those in the other species (Fig. 5; Table IV). The organization, or number of cirri along the filament (cirri/mm), also was much reduced in *Carunculina* compared to the other species. Although the shorter, less organized cirri were apparently able to intercept some *E. coli, C. texasensis* captured *E. coli* less effectively than did the other two species. The enhanced ability of the species with more complex cirri to filter *E. coli* is consistent with the hypothesis that the cirri are acting as particle-capturing structures (Owen, 1974; Owen and McCrae, 1976). This is likely to be particularly true for small (<2 μm) particles. Indeed, using laser confocal microscopy to study *in vitro* gill strips, we have observed the interaction between 0.75 μm fluorescent particles and individual cirri at a resolution approaching 0.2 μm (unpub. obs.). Morphometric analysis also indicates that when adjusted for animal dry weight, the number of cirri in a standard-sized *Corbicula fluminea* is similar to that in *Carunculina texasensis,* and is about two orders of magnitude less than that of *D. polymorpha* (Table IV). On a dry-weight basis, *D. polymorpha* had a clearance rate two orders of magnitude higher than *Carunculina texasensis* and 30 times greater than *Corbicula fluminea.*

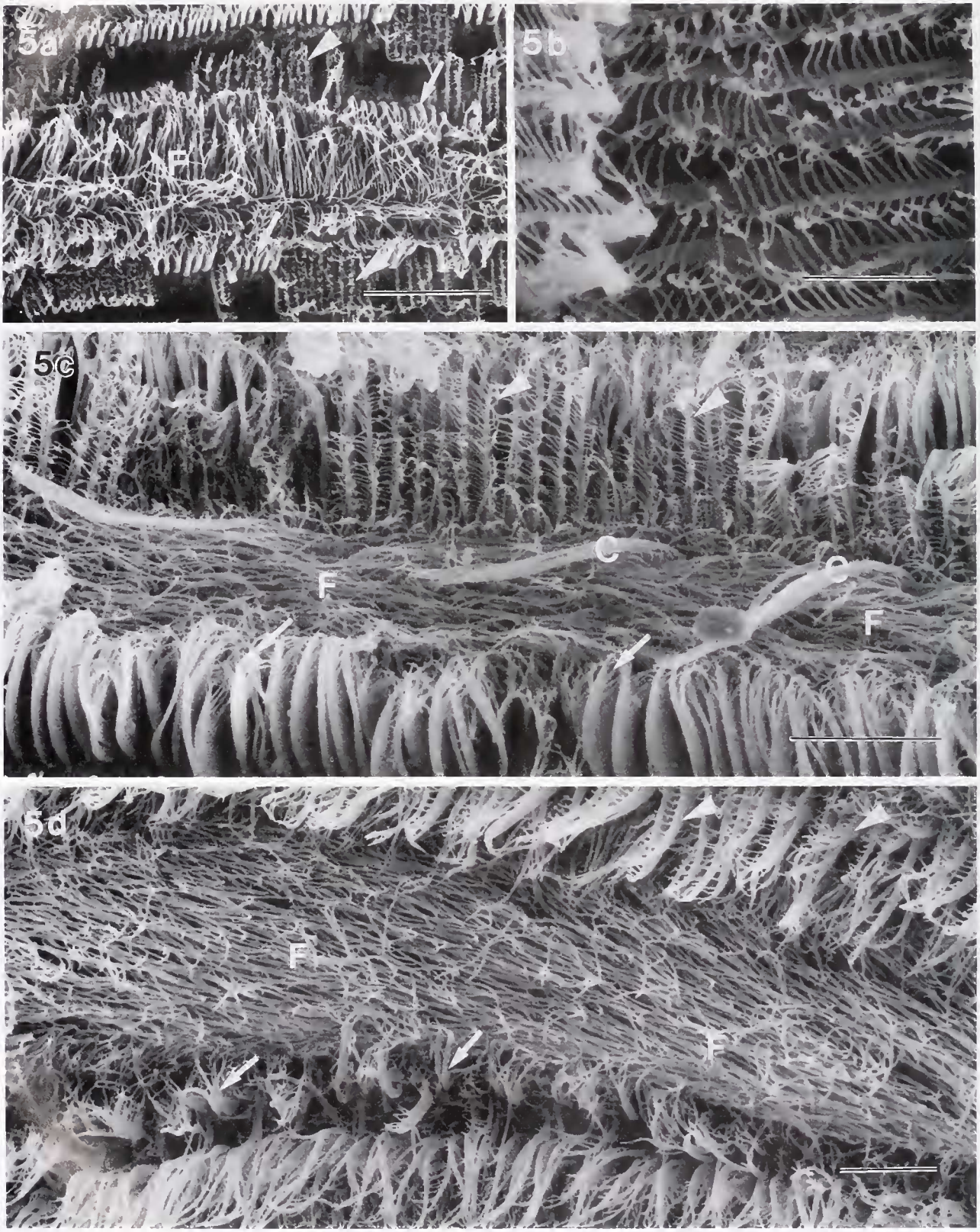

Figure 5. Scanning electron micrographs of gill filaments from *Dreissena polymorpha, Corbicula fluminea,* and *Caruncultina texasensis.* (a) Lower magnification of a *D. polymorpha* gill filament and portions of the

Table IV

Morphometric characteristics of gills from representative bivalves used in bacterial clearance studies

Bivalve species	Animal dry mass, mg	Live gill area, mm^2 per mg dry tissue	Cilia per cirri	Cirri/mm^2 dry gill	Cirri/mm^2 live gill	Cirri/mg dry tissue
Dreissena polymorpha	13.3	13.72	38–42	44,161	16,863	45,556
Corbicula fluminea	392	1.44	32–33	35,802	20,430	330
Carunculina texasensis	100	1.68	11–13	11,263	5,526	189

Many authors have shown that clearance rates of bivalves are dependent on the number of particles in the water column (Morton, 1971; Walz, 1978). Foster-Smith (1975) demonstrated that assimilation efficiency was related to the number of particles taken up by marine bivalves. The same author noted that pseudofeces are produced at particle concentrations associated with high assimilation efficiency. Walz (1978) documented a threshold of clearance below which *Dreissena* does not produce pseudofeces. We observed that no pseudofeces were produced in 30 min at bacterial concentrations that stimulated maximal particle clearance rates. This observation, coupled with our experimental pulse-washout procedures designed to minimize recycling of label, allowed us to estimate the incorporation of ^{35}S directly from bacteria. Despite the differences in filtering ability among the three species, the incorporation of ^{35}S derived from *E. coli* was similar. Even though the absolute quantity of *E. coli* ingested differed among the species, the amount of label incorporated into mussel protein per total label ingested was about 26% and did not differ among the three species.

The present study indicates that, when the results are normalized on a whole-animal basis, *D. polymorpha* and, to a lesser extent, *Corbicula fluminea* were better able to use *E. coli* than was the unionid species studied. However, and perhaps more importantly, on a dry-weight basis *D. polymorpha* was substantially better than either of the other species in clearing *E. coli* from the APW. The differences in ability to use the bacterial food source appear to correlate rather well to the different structures associated with particle capture in these species. The gill of *Dreissena* is large compared to the size of its body, and it has 138 times as many cirri as found in *Corbicula fluminea*. However, *D. polymorpha* cleared bacteria only 32 times faster than did *Corbicula fluminea*. *Corbicula* may move more water per gill surface area to achieve the observed rate of bacterial particle capture (see Way *et al.*, 1990). The differences in clearance rate described here are for a single bacterial species under laboratory conditions, and provide no information on particle selection by these bivalves. The current study also does not address the transport of particles once they are captured.

We recalculated a clearance rate from the data obtained by Reeders *et al.* (1989) for *D. polymorpha* fed a mixed population of algae. Clearance values ranged from 80 to 180 ml g^{-1} dry tissue min^{-1}; the clearance rate we found for laboratory-cultured *E. coli* was within this range. Moreover, in a study in which several marine bivalves were selected on the basis of cirri size, retention efficiencies for particles >5 μm were similar in all species (Riisgård, 1988). However, small (2 μm) particles were poorly retained by bivalves with no cirri, but species with large cirri retained similar-sized particles with 30–70% efficiency (Riisgård, 1988).

adjacent filaments. Note the presence of cirri in both the extended (arrowheads) and flexed (arrows) positions. In the flexed position the cirri tips are located over the frontal cilia (F). The cirri in *D. polymorpha* can extend across the interfilament space. Cirri from opposite sides of the filament in the fully flexed position can cover most of the frontal surface of the filament. (b) Higher magnification of the interfilament space covered by two sets of cirri from adjacent filaments. Note the "net" that is formed by the 38–42 cirri tips. (c) A gill filament from *Corbicula fluminea* showing extended cirri (arrowheads), flexed cirri (arrows), and frontal cilia (F). The filtration net formed by extended cirri is apparent. The length of the cirri indicate that in the extended position the cirri span the interfilament space. There are a large number of cilia tips (32-33) associated with a single cirri. *Corbicula fluminea* also has frontal cirri (C) located among the frontal cilia. (d) A gill filament from *Carunculina texasensis* showing cirri in the extended position (arrowheads) and in the flexed position (arrows) on opposite sides of the frontal cilia (F). Cirri from an adjacent filament at the bottom of the micrograph are in a partially flexed position. Note that individual cirri have only 12–13 free cilia tips per cirri plate (Bars: a = 20 μm; b = 5 μm; c = 10 μm; d = 10 μm).

Determination of the environmental relevance of our observations will require further study. All the bacteria used in these experiments were from laboratory strains. Bacteria in nature tend to be smaller (<1 μm) than those grown in the laboratory (see Table III), and the numbers of bacteria present in the water column will differ from environment to environment as well as from the concentrations studied here (Prieur *et al.*, 1990). However, all of the following have been documented: (1) Food particle density is an important determinant of clearance rate of bacterial-sized particles in *Dreissena*. This is not unusual and has been reported for a number of bivalves feeding on many different particles sizes, including algae, diatoms, bacteria, and detritus (Walz, 1978). (2) *Dreissena* effectively filtered all strains of laboratory-sized bacteria (1–4 μm in length). (3) The 26% efficiency of conversion of *E. coli* proteins into bivalve proteins is well within the limits associated with an energetically feasible food source. (4) The rate at which freshwater bivalves cleared laboratory bacteria was directly related to the size and number of cirri per gill surface area.

The design of this study was purposely simplified to consider only a single component of the bivalve feeding mechanism—that of particle capture or clearance. However, these experiments raise several questions about the biology of freshwater bivalves that require further study. For example, are these species able to derive significant nutrients from natural-sized bacteria in the water column during or between algal blooms? Many studies have demonstrated that bivalves use bacteria present in the water column (*e.g.*, Mikheev and Sorokin, 1966; Lucas *et al.*, 1987; Prieur *et al.*, 1990). In particular, *Mytilus edulis*, a marine species with complex cirri, rapidly clears *E. coli* from the water column in laboratory experiments in which the bacterium was presented as the sole particle source or in the presence of algae (Birbeck and McHenery, 1982; McHenery and Birbeck, 1985). Do pseudofeces deposited on the substrate serve as a nutrient resource to enhance bacterial populations in a microhabitat around freshwater bivalves as has been observed in marine bivalves (Crosby *et al.*, 1990; reviewed by Prieur *et al.*, 1990)? Thus, the potential of freshwater bivalves to use bacteria could be an important factor influencing the distribution of these bivalves and merits continued study.

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Literature Cited

Atkins, D. 1938. On the ciliary mechanisms and interrelationships of lamellibranchs. Part VII. Latero-frontal cilia of the gill filaments and their phylogenetic value. *Q. J. Microsc. Sci* **80:** 346–430.

Beninger, P. G., J. E. Ward, B. A. MacDonald, and R. J. Thompson. 1992. Gill function and particle transport in *Placopecten magellanicus* (Mollusca: Bivalvia) as revealed using video endoscopy. *Mar. Biol.* **114:** 281–288.

Beninger, P. G., S. St-Jean, Y. Poussart, and J. E. Ward. 1993. Gill function and mucocyte distribution in *Placopecten magellanicus* and *Mytilus edulis* (Mollusca: Bivalvia): the role of mucus in particle transport. *Mar. Ecol. Prog. Ser.* **98:** 275–282.

Birbeck, T. H., and J. G. McHenery. 1982. Degradation of bacteria by *Mytilus edulis*. *Mar. Biol.* **72:** 7–15.

Bunt, C. M., H. J. MacIsaac, and W. G. Sprules. 1993. Pumping rates and projected filtering impacts of juvenile zebra mussels (*Dreissena polymorpha*) in western Lake Erie. *Can. J. Fish. Aquat. Sci.* **50:** 1017–1022.

Crosby, M. P., R. I. E. Newell, and C. J. Langdon. 1990. Bacterial mediation in the utilization of carbon and nitrogen from detrital complexes by *Crassostrea virginica*. *Limnol. Oceanogr* **35:** 625–639.

Dietz, T. H., D. Lessard, H. Silverman, and J. W. Lynn. 1994. Osmoregulation in *Dreissena polymorpha*: the importance of Na, Cl, K, and particularly Mg. *Biol. Bull* **187:** 76–83.

Foster-Smith, R. L. 1975. The effect of concentration of suspension on the filtration rates and pseudofaecal production for *Mytilus edulis* L., *Cerastoderma edule* (L.) and *Venerupis pullastra* (Montagu). *J. Exp. Mar. Biol. Ecol* **17:** 1–22.

Fyhn, H. J., and J. D. Costlow. 1975. Anaerobic sampling of body fluids in bivalve molluscs. *Comp. Biochem. Physiol* **52A:** 265–268.

Jorgensen, C. B. 1976. Comparative studies on the function of gills in suspension feeding bivalves, with special reference to effects of serotonin. *Biol. Bull* **151:** 331–343.

Jørgensen, C. B. 1982. Fluid mechanics of the mussel gill: the lateral cilia. *Mar. Biol* **70:** 275–281.

Jørgensen, C. B. 1989. Water processing in ciliary feeders, with special reference to the bivalve filter pump. *Comp. Biochem. Physiol.* **94A:** 383–394.

Jørgensen, C. B., T. Kiørboe, F. Møhlenberg, and H. U. Riisgård. 1984. Ciliary and mucus-net filter feeding, with special reference to fluid mechanical characteristics. *Mar. Ecol. Prog. Ser* **15:** 283–292.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227:** 680–685.

Leach, J. H. 1993. Impacts of the zebra mussel (*Dreissena polymorpha*) on water quality and fish spawning reefs in western Lake Erie. Pp. 381–397 in *Zebra Mussels: Biology, Impacts, and Control*, T. F. Nalepa and D. W. Schloesser, eds. Lewis Publ., Boca Raton, FL.

Lucas, M. I., R. C. Newell, S. E. Shumway, L. J. Seiderer, and R. Bally. 1987. Particle clearance and yield in relation to bacterioplankton and suspended particulate availability in estuarine and open coast populations of the mussel *Mytilus edulis*. *Mar. Ecol. Prog. Ser.* **36:** 215–224.

MacIsaac, H. J., W. G. Sprules, O. E. Johannsson, and J. H. Leach. 1992. Filtering impacts of larval and sessile zebra mussels (*Dreissena polymorpha*) in western Lake Erie. *Oecologia* **92:** 30–39.

McHenery, J. G., and T. H. Birbeck. 1985. Uptake and processing of cultured microorganisms by bivalves. *J. Exp. Mar. Biol. Ecol.* **90:** 145–163.

Messing, J. 1979. A multipurpose cloning system based on the single-stranded DNA bacteriophage M13. Pp. 43–49 in *Recombinant DNA Technical Bulletin.* NIH Publication, No. 79–99, 2, Bethesda.

Mikheev, V. P., and Y. L. Sorokin. 1966. Quantitative studies of *Dreissena polymorpha* habits using the radiocarbon method. *Zhurnal Obshchey Biologii* **27:**463–472 (in Russian as cited by Morton, 1971).

Moore, H. J. 1971. The structure of the latero-frontal cirri on the gills of certain lamellibranch molluscs and their role in suspension feeding. *Mar. Biol.* **11:** 23–27.

Morton, B. 1971. Studies on the biology of *Dreissena polymorpha* Pall. V. Some aspects of filter-feeding and the effect of micro-organisms upon the rate of filtration. *Proc. Malacol. Soc. Lond. B* **39:** 289–301.

Morton, B. 1983. Feeding and digestion in bivalvia. Pp. 65–147 in *The Mollusca V. Physiology, Part 2,* A. S. M. Saleuddin and K. M. Wilbur, eds. Academic Press, New York.

Nielsen, N. F., P. S. Larsen, H. U. Riisgård, and C. B. Jørgensen. 1993. Fluid motion and particle retention in the gill of *Mytilus edulis:* video recordings and numerical modelling. *Mar. Biol.* **116:** 61–71.

Owen, G. 1974. Studies on the gill of *Mytilus edulis:* the eu-latero-frontal cirri. *Proc. R. Soc. Lond. B* **187:** 83–91.

Owen, G., and J. M. McCrae. 1976. Further studies on the latero-frontal tracts of bivalves. *Proc. R. Soc. Lond. B* **194:** 527–544.

Prieur, D., G. Mevel, J.-L. Nicolas, A. Plusquellec, and M. Vigneulle. 1990. Interactions between bivalve molluscs and bacteria in the marine environment. *Oceanogr. Mar. Biol. Annu. Rev.* **28:** 277–352.

Reeders, H. H., A. Bij de Vaate, and F. J. Slim. 1989. The filtration rate of *Dreissena polymorpha* (Bivalvia) in three Dutch lakes with reference to biological water quality management. *Freshwater Biol.* **22:** 133–141.

Richard, P. E., T. H. Dietz, and H. Silverman. 1991. Structure of the gill during reproduction in the unionids *Anodonta grandis, Ligumia subrostrata,* and *Carunculina parva texasensis. Can. J. Zool.* **69:** 1744–1754.

Riisgård, H. U. 1988. Efficiency of particle retention and filtration rate in 6 species of Northeast American bivalves. *Mar. Ecol. Prog. Ser.* **45:** 217–233.

Silvester, N. R., and M. A. Sleigh. 1984. Hydrodynamic aspects of particle capture by *Mytilus. J. Mar. Biol. Assoc. U. K.* **64:** 859–879.

Sprung, M., and U. Rose. 1988. Influence of food size and food quantity on the feeding of the mussel *Dreissena polymorpha. Oecologia* **77:** 526–532.

Stanczykowska, A., W. Lawacz, J. Mattice, and K. Lewandowski. 1976. Bivalves as a factor effecting circulation of matter in Lake Mikolajskie (Poland). *Limnologica* **10:** 347–352.

Ten Winkel, E. H., and C. Davids. 1982. Food selection by *Dreissena polymorpha* Pallas (Mollusca: Bivalvia). *Freshwater Biol.* **12:** 553–558.

Walz, N. 1978. The energy balance of the freshwater mussel *Dreissena polymorpha* Pallas in laboratory experiments and in Lake Constance. I. Pattern of activity, feeding, and assimilation efficiency. *Arch. Hydrobiol. Suppl.* **55:** 83–105.

Ward, J. E., B. A. MacDonald, R. J. Thompson, and P. G. Beninger. 1993. Mechanisms of suspension feeding in bivalves: resolution of current controversies by means of endoscopy. *Limnol. Oceanogr.* **38:** 265–272.

Way, C. M., D. J. Hornbach, C. A. Miller-Way, B. S. Payne, and A. C. Miller. 1990. Dynamics of filter-feeding in *Corbicula fluminea* (Bivalvia: Corbiculidae). *Can. J. Zool.* **68:** 115–120.

Wiegman, T., M. G. Woldring, and J. J. Pratt. 1975. A new cocktail for liquid scintillation counting of aqueous radioimmunoassay samples. *Clin. Chim. Acta* **59:** 347–356.